

that measure chemotaxis as a “yes/no” response, relative degrees of response to molecular stimuli and negative responses can also be measured within this microfluidic device. Movement of the predatory bacteria within these gradients should provide critical information about the molecules responsible for *Bdellovibrio* chemotaxis toward prey.

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Three-Dimensional Traction Force Distribution in Migrating Amoeboid Cells

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We have developed a method which enables us to determine cellular traction forces exerted perpendicular to the substrate in addition to the in-plane forces. This solution also enables to analyze the errors associated to existing two-dimensional traction cytometry methods, which assume either that the vertical displacements or that the vertical stresses are zero on the surface of the substrate. We obtain information about the substrate deformation by imaging a small volume of the elastic substrate with embedded fluorescent marker beads. Correlation with a reference image enables us to obtain the 3D deformation of the substrate. The corresponding traction forces are obtained by solving the elastostatic equation for a linearly elastic medium using the calculated deformation of the substrate. Our studies of *Dictyostelium* cells moving over flat substrates are designed to reveal the importance of various cytoskeletal components for the organization of the traction stresses in all three dimensions. We are looking at different *Dictyostelium* mutants with crosslinking defects, such as myosin II-null cells and cortaxillin-null cells, in order to study the role that these crosslinkers play in the overall distribution of the traction forces. We find that wt *Dictyostelium* cells push on the substrate near the center of the cell and pull at the periphery. The magnitude of these perpendicular forces is comparable to the magnitude of the forces produced in the plane of the substrate. Our initial findings show that the effects of mutations on the parallel forces do not necessarily predict the effects on the perpendicular forces. For example, myosin II-null cells show a significant reduction of the front to back organization of the parallel traction forces while the push pull distribution of forces remains unaffected.

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Effects of Mechanical Forces on the Cellular Dynamics

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The links between biomechanics and human diseases have been the subject of considerable scientific research effort for a number of decades. Recently, the application of soft glass rheology and polymers dynamics concepts from physics and engineering to the study of biological and physiological problems has provided valuable insights into the mechanics of cells function, their mechanical response to external stimuli and their physical and mechanical processes.

Here we report the characteristic self-similar cell stress-stiffening response of the two subsequent cytoskeleton dynamics: the early entropic and the later soft glass-like dynamics. We propose that the master relation obtained by a single parameter normalization reflects, in context with the hierarchical nature of cytoskeleton architecture, the intrinsic tight dependence of the two mechanical behaviours.

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Investigating the Mechanical Forces in Non Erythrocytic Spectrin with a New Force Sensor

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We have developed two FRET sensors, stFRET and sstFRET for studies of cell mechanics of specific proteins. These sensors permit real time monitoring of molecular forces in living cells and animals. We have previously investigated mechanical forces in cytoskeleton proteins including spectrin, actinin, filamin in HEK and BAEC cells. In these studies the FRET signal reflected the force-induced change in linker length. The maximal sensitivity for FRET and mechanical stress in a linear elastic system is linear, so to make a more efficient transducer we designed one so that force varies the angle between the donor and acceptor. In that case the dynamic range can be theoretically extended from ~ 0-100%. The new sensor is also 2/3 the size of the previous probes and is named circularly permuted sstFRET (cpstFRET). We inserted cpstFRET into non-erythrocytic spectrin and recorded the real time mechanical forces during cell migration. In the same environment cells underwent continual changes in force in spectrin during the migration with many different patterns and we should expect

varied behavior in other measures through the biochemistry. We also found spectrin bears diverse levels of force in HEK, BAEC and MDCK cell lines. To explore the mechanical linkage in cytoskeleton we treated the cells with cytochalasin D, blebbistatin and colchicine. Cytochalasin D eliminated the mechanical gradients in spectrin suggesting that the actin based network is probably the origin of the forces in spectrin. We observed that with cytochalasin the cell membrane collapsed toward the nucleus and apparently releases the tension in spectrin. Supported by the NIH.

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Polarized Fluorescence Microscopy Reveals Structural Transitions of FtsZ-Ring During Cell Division

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In Prokaryotic cells, protofilaments of FtsZ form a ring-like scaffold to constrict the cell and accomplish cell division. Understanding the structural organization of the FtsZ-ring will help to reveal the mechanism of ring contraction. In particular, the spatial orientation of FtsZ protofilaments in the cell is reported by the dipole of the fluorescent molecule rigidly attached to FtsZ. Using polarized fluorescence microscopy both in vivo and in vitro, we measure the proportion of the protofilaments oriented in the circumferential direction. We show that the Z-ring is an ordered structure during constriction, but is a disordered structure prior to the initiation of division. We measure the degree of Z-ring order as a function of Z-ring constriction radius. These results suggest that a structural transition occurs during Z-ring contraction, and provide a quantitative test for possible force generation mechanisms.

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Poisson's Ratio of a Cell Sheet Determined in Uniaxial Stretching Experiments

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Mechanical properties of living cells are strongly related to their physiological functions. In recent years, the rheology of single cells has been extensively investigated by various microbead techniques, single cell stretching and atomic force microscopy. These studies revealed that single cells exhibited a power law behavior in time and frequency domains and the power exponent value was correlated with the cytoskeletal structures. Even in the sophisticated micro and nano-rheology techniques, it cannot be determined directly the Poisson's ratio of cells, which is one of the most important physical quantities of cells. In this study, we measured the stress relaxation of a home-made cell sheet, with an uniaxial cell stretcher, to determine directly the Poisson's ratio of cells. Cell sheets of mouse fibroblast, NIH3T3 cells with ca.500 μm in length and ca.400 μm in width were fabricated by peeled off from a microfabricated substrate after the cells were confluent on the substrate for 24 h. Single stress relaxation experiments showed that the Poisson's ratio of cell sheet increased during stress relaxation with a power law behavior in a small strain and attained ca.0.20. Moreover, in repeated stress relaxation experiments, we observed that the power law exponent drastically increased while the instantaneous force and Poisson's ratio decreased as cytochalasin-D, which inhibits the polymerization of actin filaments, was added. On the other hand, adding jasplakinolide, which polymerizes the actin filaments, to samples, we observed that the power law exponent decreased on two stages, and instantaneous force and Poisson's ratio increased on one stage. The results suggest that the Poisson's ratio of cells increases with stabilizing the actin filamentous structures.

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Motor Properties of Molecular Spiders

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Molecular spiders are synthetic molecular motors featuring multiple legs such that each leg can interact with a substrate through binding and cleavage. Experimental studies of molecular spiders suggest that the motion of the spider on both a substrate matrix [R. Pei *et al.*, *J. Amer. Chem. Soc.* **128**, 12693 (2006)] and a two dimensional substrate track [K. Lund *et al.*, *Nature* **465**, 206 (2010)] is biased towards uncleaved substrates. We first investigated the origin of the spider's biased motion by using Monte Carlo simulations of bipedal spiders on a 1D track based on a realistic chemical kinetic model [L. Samii *et al.*, *Phys. Rev. E* **81**, 021106-1 (2010)], and found that substrate cleavage and spider detachment from the track both contribute to biased motion of the spider population. In the work reported here, we extend these studies by investigating how experimental parameters such